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The relationship between combat-related traumatic amputation and subclinical cardiovascular risk

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ABSTRACT

Background: The relationship between acute combat-related traumatic injury (CRTI) to coronary flow reserve (CFR) and subclinical cardiovascular risk have not been examined and was the primary aim of this study.
Methods and results: UK combat veterans from the ADVANCE cohort study (UK-Afghanistan War 2003–14) with traumatic limb amputations were compared to injured non-amputees and to a group of uninjured veterans from the same conflict. Subclinical cardiovascular risk measures included fasted blood atherogenic index of plasma (AIP), triglyceride-glucose index (TyG; insulin resistance), the neutrophil-lymphocyte ratio (NLR) and high-sensitivity C-reactive protein (hs-CRP; vascular inflammation), body mass index (BMI) and visceral fat volume (dual-energy X-ray absorptiometry) and 6-min walk distance (6MWD; physical performance). The sub-endocardial viability ratio (SEVR), to estimate CFR, was calculated using arterial pulse waveform analysis (Vicorder device). In total 1144 adult male combat veterans were investigated, comprising 579 injured (161 amputees, 418 non-amputees) and 565 uninjured men. AIP, TyG, NLR, hs-CRP, BMI, total body fat and visceral fat volume were significantly higher and the SEVR and 6MWD significantly lower in the amputees versus the injured-non-amputees and uninjured groups. The SEVR was lowest in those with above knee and multiple limb amputations. CRTI (ExpB 0.96; 95% CI 0.94–0.98: $p < 0.0001$) and amputation (ExpB 0.94: 95% CI 0.91–0.97: $p < 0.0001$) were independently associated with lower SEVR after adjusting for age, rank, ethnicity and time from injury.
Conclusion: CRTI, traumatic amputation and its worsening physical deficit are associated with lower coronary flow reserve and heightened subclinical cardiovascular risk.

1. Introduction

There is increasing evidence to support the association between combat-related traumatic injury (CRTI) and CVD risk. A recent systematic review has shown that the majority of supportive data relates to cross-sectional or retrospective studies relating to historical conflicts and they lacked an uninjured comparator group leading to significant bias. [1] Nevertheless, the body of evidence in support of the association appears to be growing. [2,3] Currently, the most convincing evidence relates to the cardiovascular consequences of traumatic limb amputation, which has been linked to an increased risk of obesity, dyslipidaemia, hypertension and cardiovascular death. [1,3,4]

There is a clearly a need for contemporary data relating to the impact of CRTI on CVD risk in relation to recent military conflicts in order to determine whether this still holds true. This is important as modern improvements in personal protective equipment and post-injury medical/surgical care and rehabilitation could have positively influenced the cardiovascular trajectory of the injured. [5,6] These advances have led

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to the survival of injured combatants who would almost certainly have died if exposed to similar injuries only two decades previously. One of the legacies of the recent wars in Iraq and Afghanistan has been the large number of surviving combat veterans with significant physical injuries for whom the cardiovascular consequences remain unknown.

The measurement of novel blood biomarkers could provide an earlier understanding of the CRTI-cardiovascular risk relationship. Their predictive precision may be further enhanced by combing ≥2biomarkers into a single composite marker. This approach could provide a more in-depth understanding of the pathophysiological and potentially causal effects of CRTI. The atherogenic index of plasma (AIP, atherogenesis), triglyceride-glucose (TyG) index (insulin resistance) and the neutrophil-lymphocyte ratio (vascular inflammation) are three established examples [7–9]. They have all been independently linked to worsening coronary artery disease (CAD) and major adverse cardiovascular events (MACE). [10–14]

The non-invasive measurement of the subendocardial viability ratio (SEVR), using application tonography, is another novel biomarker of subclinical cardiovascular disease risk. It is surrogate marker of myocardial perfusion to left ventricular (LV) workload matching and is strongly correlated with coronary microcirculatory function and coronary flow reserve. [15] [16].

The relationship between CRTI and traumatic amputation and its severity on the SEVR has not been explored and was investigated for the first time in this study. We further sought to examine the influence of CRTI and traumatic amputation on novel composite subclinical biomarkers of inflammation (NLR), atherogenesis (AIP) and insulin resistance (TyG).

2. Materials and methods

2.1. Study population

The population used in this study was recruited from the baseline survey of the on-going ADVANCE Prospective Cohort study. The ADVANCE Study consists of 579 adult (≥18 years) British military servicemen who sustained CRTI (sufficient to require aeromedical evacuation and admission to UK hospital) during recent military operations in Afghanistan (2003–2014). A frequency matched group (by age, sex [all men], service, rank, regiment, deployment period and role-in-theatre to the injured group) of 565 uninjured combat veterans are compared to the injured group; the detailed study design, protocol and sample size calculation have been previously published. [17] Participants with established CVD, including a history of stroke, transient ischaemic attack, ischaemic heart or peripheral vascular disease, prior to their injury/deployment of interest, or evidence of active acute infection at baseline survey were excluded. [17]

Study participation was voluntary and followed full written informed consent. Ethical approval was granted by the MoD Research and Medical Ethics Committee (MoDREC: 357PPE12) and this study complied with the principles outlined in the Declaration of Helsinki.

2.2. Data collection

All clinical data were collected at a single baseline study visit conducted over approximately six hours and led by trained research nurses. The participants were asked to fast and refrain from caffeine and alcohol for at least eight hours prior to all venous blood tests which were sent to the local NHS laboratory for blind processing. Blood tests included high sensitivity C-reactive protein (hs-CRP), fasting glucose and lipids, renal function and full blood count. Collected information and demographics included confirmation of the participant's ethnicity, medical and family history (of stroke or IHD) and smoking status, measured height, abdominal waist circumferences (measured manually using a tape measure). Body mass index (MBI) was calculated as weight (kg)/height $(m)^2$ with body weight being adjusted in amputees using an established correction formulae to account for the mass of their missing limb(s). [18] Military ranks were classified into three recognised categories as previously described [2,19]: senior rank (commissioned officers), mid rank (senior non-commissioned officers) and junior rank (junior non-commissioned officers and other lower ranks). Injury severity was quantified using the New Injury Severity Score (NISS). [17] The NISS ranges from 1 to 75 and is calculated using the sum of squares of the three most severe injuries irrespective of body region injured. [20]

2.3. Primary outcome - subendocardial viability ratio

The SEVR was measured from pulse waveform analysis of the brachial arterial pressure using the Vicorder device (Skidmore Medical, UK). [21,22] Measurements were undertaken by trained research nurses in a temperature-controlled room after the participants had rested for five minutes, lying supine with their head raised to 30°. [21,23] The SEVR was automatically calculated by the Vicorder software as the diastolic pressure-time index (DPTI) divided by systolic pressure time index (SPTI). Diastolic pressure-time index is the area between aortic and left ventricular end-diastolic pressure (LVEDP) curves during diastolic time (DT), whereas systolic pressure-time index is the area under aortic pressure curve during systolic ejection period (SEP). All Vicorder measures were done in at least triplicate and the median value was used.

Reproducibility was excellent with the median coefficient of variation for ≥3 repeat SEVR readings for the entire cohort being 5.12% (interquartile range [IQR] 3.15–8.16%). Resting mean arterial blood pressure and total peripheral resistance were also measured using the Vicorder.

2.4. Novel composite biomarkers

The AIP analytically calculated as log (triglycerides/HDL-C) using molar concentrations (mmol/l) of triglycerides and HDL-C. [7] The TyG index was calculated as ln [fasting triglyceride (mg/dl) × fasting glucose (mg/dl)]/2 [12]. The NLR was calculated as the ratio of the neutrophil count / lymphocyte count. [9]

2.5. Amputee status, body composition assessment and physical fitness

Participants with knee disarticulation and trans-femoral limb amputation were classified as above knee amputees and the rest as below knee/other (eg upper limb) amputations. Percentage body fat and visceral fat volume were measured using dual-energy X-ray absorptiometry (DXA, Vertec Horizon and Discovery, UK) [17]. Physical fitness and exercise burden were calculated using the long-form of the International Physical Activity Questionnaire (IPAQ). [24,25] The total weekly burden of exercise was quantified as the total weekly minutes of exercise undertaken for each of three exercise domains (walking, moderate and vigorous exercise). The results were quantified in METS (metabolic equivalent) in which the total weekly minutes of each exercise domain (minutes per day x days per week undergoing the exercise) were weighted (x3.3 for walking, x4 for moderate exercise and x8 for vigorous exercise) and the total used to represent the total exercise burden. All walking, moderate and vigorous time variables were truncated to a maximum of 180 min per day for each modality as previously described. [24] Physical-function was measured using the six-minute walk distance (6MWD) test. [17].

2.6. Statistical analysis

Continuous data were presented as means (± standard deviation [SD]) for normally distributed data and medians (IQR) for skewed data. Unpaired *t*-tests or Mann-Whitney *U* tests, as appropriate, were used in two-group comparisons of continuous data; three-group comparisons were made using one-way ANOVA or Kruskal-Wallis tests. The chi-squared or Fisher's exact tests were used to compare categorical data.

Correlations were measured using Spearman rank coefficients (95% CI).

The relationship between CRTI, amputation and amputation type (single vs multiple and above versus below knee/other amputation) and log SEVR were examined using multivariable linear regression with robust standard errors. The coefficients from the regression model were exponentiated and reported as Geometric Mean Ratios (GMR). The model was adjusted 'a priori' for age at assessment (years), ethnicity (Caucasian vs other), time from injury/deployment (years) and military rank (by three groups: officer, middle and junior). [2]

A two-tailed p value <0.05 was considered statistically significant. Statistical analyses were undertaken with SPSS 26.0 (SPSS, Chicago, IL, USA) and GraphPad Prism version 6.07 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

In total 1144 combat veterans were investigated, comprising 579 men with CRTI and 565 uninjured men. Among the injured there were 418 injured-non-amputees and 161 amputees of whom there were 86 single and 75 double or triple amputees; there were 92 above knee amputations and 69 below knee / other amputees. The amputees were marginally yet significantly younger and their time from injury/deployment was less than the injured non-amputees and the uninjured (Table 1). The amputees were of lower rank, had a greater proportion of blast injuries, had a higher median NISS and were more likely to have left the military than the injured non-amputees and uninjured (Table 1). Complete SEVR data were available on 1111 participants (97.1%).

Table 1
Baseline demographics among the uninjured versus the combat-related traumatic injury (CRTI) participants.

	Uninjured	Injured		
		Non Amputees	Amputees	P value
Number	565	418	161	–
Age at assessment, years	34.25 ± 5.41	34.41 ± 5.56	32.98 ± 4.60	0.012
Time from deployment/injury to assessment, years	8.21 ± 2.15	8.62 ± 2.16	7.57 ± 1.92	<0.001
Left military service	99 (17.5%)	277 (66.3%)	144 (89.4%)	<0.001
Rank/NS-SEC				
EC (at sampling)	79 (14.0%)	46 11.0%)	13 (8.1%)	
-Senior rank	147 (26.0%)	86 (20.6%)	20 (12.4%)	
-Mid rank	339 (60.0%)	1286 (68.4%)	128 (79.5%)	<0.001
-Lower rank				
Injury mechanism				
-Blast	-	283 (67.7%)	152 (94.4%)	
-Other (accidents, gunshot, burns)	-	135 (22.3%)	9 (15.6%)	<0.001
New Injury Severity Score, median (IQR)	–	9.0 (4.0–17.0)	25.0 (17.0–34.0)	<0.001
Ethnicity				
-Caucasian	512 (90.6%)	376 (90.0%)	148 (91.9%)	
-Other (including mixed race)	53 (9.4%)	42 (10.0%)	13 (8.1%)	0.770
Family history of CVD[§]	111 (19.6%)	69 (16.5%)	37 (23.0%)	0.174
Smoking history				
-Current smoker	126 (22.3%)	90 (21.5%)	29 (18.0%)	
-Ex smoker	178 (31.5%)	112 (26.8%)	56 (34.8%)	0.226
-Never Smoked	261 (46.2%)	216 (51.7%)	76 (47.2%)	

[§] First degree relative; CVD, cardiovascular disease; NS-SEC, national statistics socioeconomic classification; IQR, inter-quartile range.

Body mass index, waist/height ratio, whole body fat % and visceral fat volume were significantly higher in the amputees versus the uninjured and injured non-amputees (Table 2). The amputees also had significantly higher resting heart rate, triglycerides, AIP, TyG, NLR and hs-CRP levels and lower HDL-C, SEVR and 6MWD versus the injured-non-amputees and uninjured groups (Tables 2 and 3). SEVR was significantly lower in the above knee (186.3 ± 33.60; $p < 0.001$) and multiple limb amputees (187.6 ± 32.07; $P < 0.001$) compared with below knee/other (192.7 ± 41.08) and single amputees (190.3 ± 40.92), injured non-amputee (195.5 ± 37.6) and uninjured groups (202.5 ± 41.07) (Figs. 1 and 2).

Six-minute walk distance and total weekly exercise were positively correlated with SEVR (Table 4). BMI, waist-height ratio, whole body fat %, visceral fat volume, resting heart rate, mean brachial artery blood pressure, triglycerides, TyG index, NLR, hs-CRP and glucose were inversely correlated with SEVR. Whilst statistically significant, with the exception of resting heart rate ($r = -0.75$), all of these correlations were weak (Table 4).

CRTI (yes vs no) and traumatic limb amputation were associated with lower SEVR (Table 4). After adjustment for confounders (age, time from injury/deployment, ethnicity and rank) this association remained. CRTI was independently associated with a 4% (95% CI: 1–6%) reduction

Table 2
Comparative anthropometric indices and venous blood results of the uninjured versus the combat-related traumatic injury (CRTI) participants.

	Uninjured	Injured		
		Non-Amputees	Amputees	P value
Number	565	418	161	–
Body Mass Index, kg/m^2	27.43 ± 3.39	27.89 ± 3.69	28.77 ± 4.40	<0.001
Waist/height ratio	0.52 ± 0.06	0.53 ± 0.05	0.54 ± 0.06	0.002
Whole body fat, %	25.65 ± 4.75	26.44 ± 5.40	27.65 ± 5.83	<0.001
Visceral fat volume, mm^3	471.8 ± 179.7	509.6 ± 211.2	554.1 ± 229.6	<0.001
Heart rate, beats/min	56.30 ± 8.38	58.62 ± 9.39	63.24 ± 10.74	<0.001
Mean brachial arterial blood pressure, mmHg	94.68 ± 9.68	94.84 ± 9.46	94.29 ± 10.68	0.835
Total peripheral resistance	0.92 ± 0.22	0.91 ± 0.18	0.91 ± 0.19	0.479
Subendocardial viability ratio	202.5 ± 41.07	195.5 ± 37.6	189.6 ± 40.5	0.005
Glucose, mmol/l	4.95 ± 0.66	5.02 ± 1.34	4.95 ± 1.25	0.561
Glycated haemoglobin, HbA1c	34.64 ± 3.74	34.93 ± 9.06	34.13 ± 6.37	0.416
LDL cholesterol, mmol/l	3.11 ± 0.81	3.05 ± 0.87	3.02 ± 0.77	0.334
Triglycerides, mmol/l	1.10 (0.80–1.50)	1.10 (0.8, 1.60)	1.20 (0.8–1.80)	0.047
HDL cholesterol, mmol/l	1.31 ± 0.30	1.30 ± 0.32	1.15 ± 0.28	<0.001
Atherogenic index of plasma (AIP)	−0.07 (−0.22,1.10)	0 (−0.21, 0.19)	−0.03 (−0.22, 0.21)	0.004
Triglyceride-glucose (TyG) Index	6.82 ± 0.54	6.85 ± 0.56	6.92 ± 0.54	0.138
Neutrophil-lymphocyte ratio	1.63 ± 0.66	1.65 ± 0.69	1.87 ± 0.86	<0.001
Hs-CRP, mg/l	0.85 (0.50, 1.76)	0.90 (0.47, 1.90)	1.30 (0.61, 2.80)	<0.001
Six minute walk distance, m	630.6 ± 95.69	576.4 ± 150.8	421.7 ± 202.1	<0.001
Total exercise, Mets per week	7332 (3684, 13,236)	6716 (3156, 12,496)	6960 (3168, 15,510)	0.212

Hs-CRP, high-sensitivity C reactive protein; LDL, low density lipoprotein; HDL, high density lipoprotein; P values refer to the overall comparison between amputees, injured-non amputees and the uninjured groups.

Table 3
Correlations between variables and Subendocardial Viability ratio.

	Correlation coefficient (95% CI)	*P* value
Age	−0.05 -(0.11, 0.007)	0.076
Body mass index	−0.15 (−0.21, −0.09)	<0.001
Waist/height ratio	−0.16 (−0.22, −0.10)	<0.001
Whole body fat %	−0.25 (−0.30, −0.19)	<0.001
Visceral fat volume	−0.22 (−0.289, −0.16)	<0.001
Resting heart rate	−0.73 (−0.79, −0.66)	<0.001
Mean brachial artery blood pressure	−0.15 (−0.21, −0.09)	<0.001
HDL-C	0.12 (0.05, 0.18)	<0.001
Triglycerides	−0.15 (−0.21, −0.09)	<0.001
AIP	−0.17 (−0.23, −0.11)	<0.001
TyG index	−0.15 (−0.21, −0.09)	<0.001
Neutrophil-lymphocyte ratio	−0.15 (−0.21, −0.09)	<0.001
Hs-CRP	−0.13 (−0.19, −0.07)	<0.001
Six-minute walk distance, m	0.09 (0.04, 0.16)	<0.001
Total weekly exercise, Mets	0.08 (0.02, 0.14)	0.007

CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; HS-CRP, high-sensitivity C-reactive protein; AIP atherogenic index of plasma

in SEVR compared to the uninjured. Amputee status was also independently associated adjusted for age, time from injury/deployment, ethnicity and rank) with decreased SEVR: the injured-non amputees had a 3% (CI 1–5%) reduction and amputees a 6% (CI 3–9%) reduction in SEVR compared to the uninjured group. Above knee and multiple limb amputees were the strongest independent amputee-related associates of decreased SEVR (Table 5).

4. Discussion

In this study we examined the relationship between CRTI, and traumatic limb amputation, and SEVR and other novel composite biomarkers of cardiovascular risk. We found that SEVR was significantly lower in participants with CRTI and a history of limb amputation compared to those who were uninjured. CRTI, traumatic limb amputation and its worsening severity (above knee and multiple limb amputation) were independently associated with lower SEVR. Limb amputation was also associated with significantly higher AIP (atherogenesis), systemic inflammation (hs-CRP and SII), insulin resistance (TyG), relative obesity (BMI, waist/height ratio, visceral volume and whole body fat %) and lower physical performance (6MWD).

In this study we observed the lowest SEVR values to be in amputees, which is a novel finding. Lower SEVR is a surrogate marker of one or both of lower myocardial (and coronary) blood flow and/or increasing oxygen demand. The observation that resting heart rates were significantly higher in the amputees may be an indicator of greater myocardial oxygen demand; a reduction in myocardial perfusion would be more difficult to infer. Indeed, there were no differences in total peripheral resistance and mean arterial blood pressure linked to increased left ventricular afterload and reduced coronary flow reserve. However, we cannot exclude microvascular adaptations and changes in coronary endothelial dysfunction that have been linked to reduced myocardial blood flow. [26] In a previous publication from the ADVANCE study we reported an independent association between increasing severity of CRTI and both metabolic syndrome and arterial stiffness in the absence of any changes in central or brachial systolic blood pressure. [2] It is interesting that the SEVR was lower in the amputees despite amputees being significantly younger than the uninjured amputees and uninjured. SEVR tends to be higher in younger versus older adults. [27,28] SEVR was inversely related to age in our study; this inverse relationship was significant on multiple regression analysis after adjustment for confounders.

The independent effect of CRTI and amputation on SEVR whilst statistically significant was modest and generally associated with a < 10% reduction in SEVR. The inverse, albeit weak, correlations between markers of inflammation (NLR and hs-CRP), atherogenesis (AIP), insulin resistance (TyG), relative obesity (visceral fat volume, BMI, waist/height and whole body fat) and lower exercise and 6MWD in our study support the existing literature; increased markers of cardiovascular risk are known to be associated with lower SEVR. [26,29,30]

The atherogenic index of plasma (AIP) has been widely reported to be a surrogate marker of atherosclerosis. In a recent systematic review and meta-analysis that included 14 articles and 40,902 participants from seven different countries it was shown that a one-unit increase in AIP was associated with higher odds of developing CAD (OR 2.11; 95% CI 1.65–2.69; $P < 0.001$; $I^2 = 98\%$). [13] In a very recent cohort study, not included in the above meta-analysis, Sadeghi et al. examined 6323 healthy adults over 15 years. They found that AIP was independently associated with increased MACE [10]. This could suggest that the higher triglycerides and lower HDL (ie higher AIP) among the amputees represents an early relative marker for CVD risk compared with the injured non amputees and uninjured. It is worth noting that whilst the exact AIP

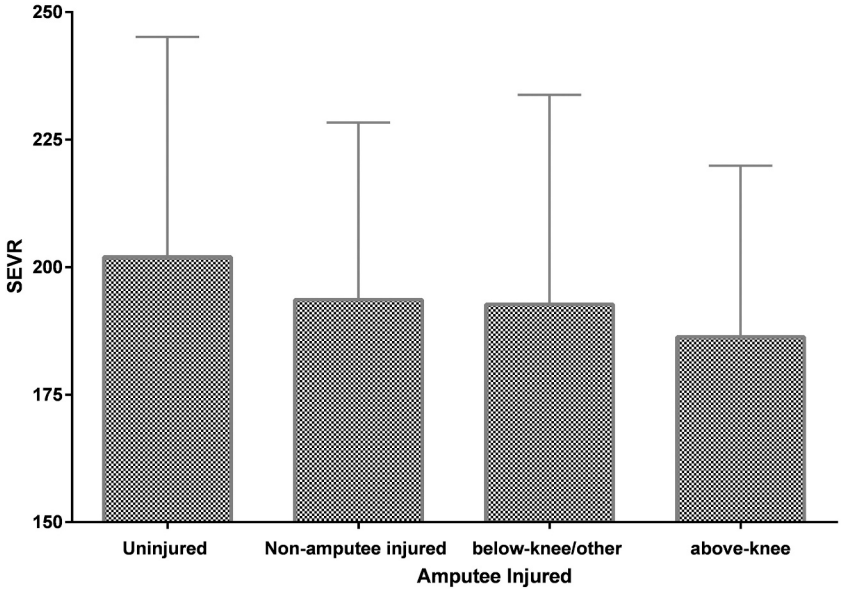

Fig. 1. Comparison of Subendocardial viability ratio among the uninjured (n = 549), injured non-amputees (n = 407) and amputee by amputation level (67 below knee and 89 above knee amputees); Results are shown as mean ± SD.

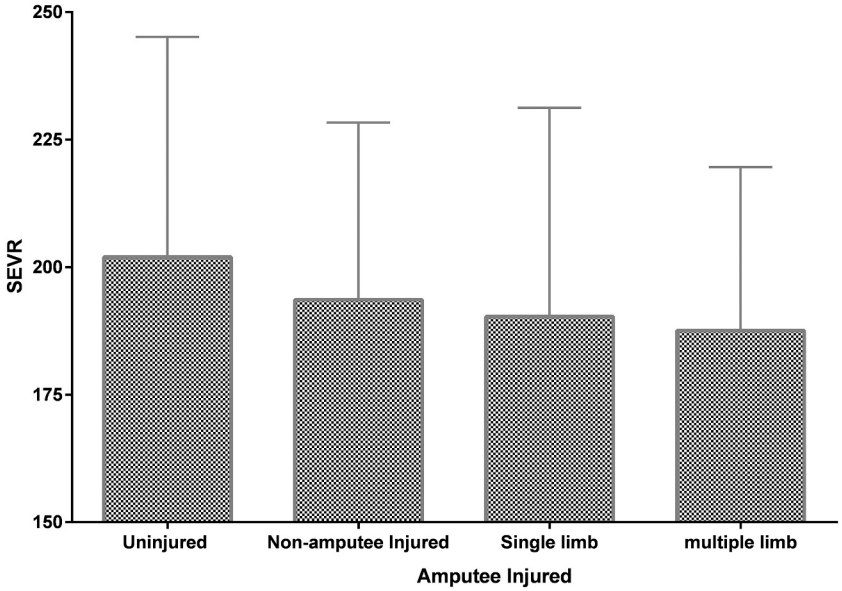

Fig. 2. Comparison of Subendocardial viability ratio among the uninjured ($n = 549$), injured non-amputees ($n = 407$) and amputee by amputation number (84 single and 72 multiple limb amputees); Results are shown as mean ± SD.

Table 4
Influence of Traumatic injury and limb amputation on outcome variable of subendocardial viability ratio using multiple linear regression.

	Univariable		Multivariable			
			Model 1 CRTI		Model 2 Amputation	
	Unadjusted GMR (95% CI)	P value	Adjusted GMR (95% CI)	P value	Adjusted GMR (95% CI)	P value
Uninjured (ref)	1.00 (ref)		-			
-Injured (CRTI)	0.95 (0.93–0.97)	<0.001	0.96 (0.94–0.98)	<0.001	-	
Uninjured (ref)	1.00 (ref)	-	-	-	1.00 (ref)	-
-Injured non-amputee	0.96 (0.94–0.98)		-	-	0.97 (0.95–0.99)	-
-Injured amputee	0.94 (0.91–0.97)	<0.001	-	-	0.94 (0.91–0.97)	<0.001
Age at assessment	0.99 (0.99–1.00)	0.089	0.99 (0.98–0.99)	0.003	0.99 (0.98–0.99)	0.004
Caucasian	1.02 (0.99–1.06)	0.230	1.0 (0.97–1.04)	0.993	1.0 (0.97–1.04)	0.952
Time from injury, years	0.99 (0.98–0.99)	0.003	0.99 (0.98–0.99)	0.048	0.99 (0.98–0.99)	0.028
NS-SEC/Rank						
-Officer rank (NS-SEC 1)	1.00 (ref)		1.00 (ref)		1.00 (ref)	
-Mid rank (NS-SEC 2)	0.96 (0.92–0.99)		0.97 (0.93–1.01)		0.97 (0.93–1.01)	
-Junior rank (NS-SEC 3)	0.93 (0.90–0.97)	<0.001	0.92 (0.88–0.96)	<0.001	0.92 (0.89–0.96)	<0.001

CRTI, combat-related traumatic injury; NS-SEC, National Statistics Socio-economic classification; GMR, Geometric mean ratio; Ref, reference category; Each model has been adjusted for age at assessment, ethnicity, rank, time from injury and ethnicity.

level used to identify higher risk individuals vary by the population studied the AIP levels in the amputee groups were still well below the typical higher risk cut-off of ≥0.2. [7,10,11]

This is similarly true for the TyG index where again the mean TyG levels even in the amputee group would not be indicative of high risk. However, as with AIP, the relationship between TyG and CVD is relatively linear. Hence the higher TyG levels in the amputees would suggest higher 'relative' insulin resistance versus the injured non-amputees and uninjured groups. The results of a very recent meta-analysis of 13 observational studies of 49,325 participants showed that TyG index's pooled area under the receiver-operating characteristics was 0.87 for a diagnosis of MetS (CI 95: 0.84–0.90). [31] Interestingly, TyG index was found to be superior to the homeostasis model assessment of insulin resistance (HOMA-IR), which is the commonest described measure of insulin resistance, for predicting MetS. [32] It is notable that both serum glucose and HbA1c levels were similar in the amputee and non-amputee groups suggesting that the differences in TyG levels have not translated into demonstrable differences in glycaemic control.

The greater NLR and hs-CRP in the amputees versus the injured non-amputees and uninjured groups suggests the presence of greater background systemic inflammation. The greater relative obesity measures in the amputee versus the other the other two groups offer potential pathogenic insights. Obesity and in particular elevated visceral fat leads to increased production of inflammatory cytokines (eg tumour necrosis factor α and interleukin 6) and reduced production of adiponectin, which is an anti-atherogenic, anti-diabetic, and anti-inflammatory protein [33]. We observed significantly greater BMI, waist/height ratio, total body fat % and visceral fat volume in the amputees compared the other two groups supporting this potential mechanism.

Despite the fact that the amputees reported a similar amount of weekly exercise to the injured non-amputees they had the worst physical function in terms of the 6MWD. Lower physical functioning in the amputees is not unexpected given the large number of above knee and multi-limb amputees within our cohort. The 6MWD is a highly objective measure of physical functioning that has been shown to be repeatable and responsive to changes in clinical status. [34] Lower 6MWD is known to be associated with obesity, increased vascular inflammation and increased CVD risk and endorsed by our data. [35,36] It is difficult to know whether the relative greater exercise burden observed in the

Table 5
Influence of Amputation type and number on dependent outcome of subendocardial viability ratio using multiple linear regression.

	Univariable		Multivariable			
			Model 1 Amputation level		Model 2 Amputation number	
	Unadjusted GMR (95% CI)	P value	Adjusted GMR (95% CI)	P value	Adjusted GMR (95% CI)	P value
Uninjured (ref)	1.00 (ref)		1.00 (ref)			
-Injured non amputee	0.96 (0.94–0.98)	-	0.97 (0.95–0.99)	-		
-Above knee amputation	0.92 (0.884–0.96)		0.96 (0.92–1.00)	-		
-below knee/other amputation	0.95 (0.91–0.96)	<0.001	0.92 (0.89–0.96)	<0.001		
Uninjured (ref)	1.00 (ref)				1.00 (ref)	
-Injured non amputee	0.96 (0.94–0.98)				0.97 (0.95–0.99)	
-single limb amputation	0.94 (0.90–0.98)				0.95 (0.91–0.99)	
-multiple limb amputation	0.94 (0.89–0.98)	0.004			0.93 (0.89–0.98)	<0.001
Age at assessment	0.99 (0.99–1.00)	0.089	0.99 (0.98–0.99)	0.004	0.99 (0.98–0.99)	0.007
Caucasian	1.02 (0.99–1.06)	0.230	1.0 (0.97–1.04)	0.978	1.0 (0.96–1.04)	0.993
Time from injury, years	0.99 (0.98–0.99)	0.003	0.99 (0.98–0.99)	0.048	0.99 (0.98–0.99)	0.009
NS-SEC/Rank, at sampling						
-Officer rank (NS-SEC 1)	1.00 (ref)		1.00 (ref)		1.00 (ref)	
-Mid rank (NS-SEC 2)	0.96 (0.92–0.99)		0.97 (0.93–1.01)		0.96 (0.93–1.00)	
-Junior rank (NS-SEC 3)	0.93 (0.90–0.97)	<0.001	0.92 (0.88–0.96)	<0.001	0.92 (0.89–0.95)	<0.001

CRTI, combat-related traumatic injury; NS-SEC, National Statistics Socio-economic classification; GMR; Geometric mean ratio; Ref, reference category; Each model has been adjusted for age at assessment, ethnicity, rank, time from injury and ethnicity.

amputees is a genuine behavioural modification in response to their injuries and could act to mitigate some of their risk. In this study the long-form of the IPAQ questionnaire was used, which is known to be highly subjective. Despite being one of the gold standard exercise questionnaires the correlation between the IPAQ and objective measures of activity or fitness has been shown to be modest, at best, with a tendency to overestimate physical activity. [25,37] The variability appears to be even greater in the long form of IPAQ used in this study.

This study has a number of additional limitations that should be acknowledged. All of the biomarkers examined are known to be indirect and surrogate markers of CVD risk and our reported associations do not necessarily imply causation. Nevertheless, causal inferences can be postulated and have been based on sound scientific merit using validated biomarkers. Whilst the injured group in ADVANCE was frequency-matched to the uninjured group we undertook an additional analysis of the amputees and non-amputees within the injured group and compared them to the uninjured. The amputees were younger with a greater proportion of lower ranks represented. This could be potential source of bias as lower rank is known to equate to lower socioeconomic class which itself is linked to increased cardiovascular risk. [2,19,38] However, the fact that CRTI, traumatic limb amputation and its worsening physical deficit (above knee and multiple amputees) were all independently linked to lower SEVR, even after adjusting for age and rank is supportive of findings.

In conclusion, in this baseline analysis of the ADVANCE Cohort we found that CRTI and associated limb amputation were associated with lower SEVR and increased subclinical cardiovascular risk. This increased subclinical risk profile was typified by relatively greater levels of biomarkers of atherosclerosis (AIP), systemic inflammation (hs-CRP and NLR), insulin resistance (TyG) and obesity (BMI, visceral and total body fat). CRTI, traumatic amputation and worsening amputation-related injury (above knee and multiple amputations) were independently associated with lower SEVR suggestive of lower coronary flow reserve. These findings need to be confirmed with longitudinal data in this cohort.

Contributors

Conceptualization (CJB, PC, NTF, AMJB, ANB); Data curation (CJB, SS, ANB); Formal analysis (CJB, SS); Funding acquisition (CJB, PC, NTF, AMJB, ANB); Investigation (CJB, PC, NTF, AMJB, ANB, SS); Methodology (CJB, SS, AMJB, NTF, ANB); Project administration (ANB); Resources, Software, Supervision, Validation, Visualization (CJB, SS, PC, AMJB, NTF, ANB).

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Author statement

We declare that our manuscript represents original research and has not been previously published or submitted to another journal. The authors so not have any potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. The protocol paper for the ADVANCE Study was published in BMJ Open in 2020. All authors have approved the final version of the manuscript and have no conflict of interest to declare.

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Declaration of Competing Interest

The authors have no competing interests to declare.

Data availability

Owing to the sensitive nature of this study, data access requests will be carefully considered on a case by case basis by the ADVANCE study project board and UK Ministry of Defence.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2023.131227.

References

[1] C.J. Boos, N. De Villiers, D. Dyball, A. McConnell, A.N. Bennett, The relationship between military combat and cardiovascular risk: a systematic review and Meta-analysis, Int. J. Vasc. Med. 2019 (2019) 9849465.
[2] C.J. Boos, S. Schofield, P. Cullinan, D. Dyball, N.T. Fear, A.M.J. Bull, et al., Association between combat-related traumatic injury and cardiovascular risk, Heart 108 (2022) 367–374.
[3] K.B. Tavares, D.M. Russell, R.J. Conrad, G.C. Sizemore, S.H. Nguyen, A.Y. Moon, et al., Time to weigh in on obesity and associated comorbidities in combat-wounded amputees, J. Trauma Acute Care Surg. 90 (2021) 325–330.
[4] J.E. Naschitz, R. Lenger, Why traumatic leg amputees are at increased risk for cardiovascular diseases, QJM : Month. J. Assoc. Phys. 101 (2008) 251–259.
[5] J.T. Howard, R.S. Kotwal, C.A. Stern, J.C. Janak, E.L. Mazuchowski, F.K. Butler, et al., Use of combat casualty care data to assess the US military trauma system during the Afghanistan and Iraq conflicts, 2001-2017, JAMA Surg. 154 (2019) 600–608.
[6] J. Etherington, A.N. Bennett, R. Phillip, A. Mistlin, Outcomes for UK service personnel indicate high quality trauma care and rehabilitation, Bmj. 354 (2016), i4741.
[7] J.C. Fernández-Macías, A.C. Ochoa-Martínez, J.A. Varela-Silva, I.N. Pérez-Maldonado, Atherogenic index of plasma: novel predictive biomarker for cardiovascular illnesses, Arch. Med. Res. 50 (2019) 285–294.
[8] V.K. Ramdas Nayak, P. Satheesh, M.T. Shenoy, S. Kalra, Triglyceride glucose (TyG) index: a surrogate biomarker of insulin resistance, J. Pak. Med. Assoc. 72 (2022) 986–988.
[9] A. Buonacera, B. Stancanelli, M. Colaci, L. Malatino, Neutrophil to lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases, Int. J. Mol. Sci. (2022) 23.
[10] M. Sadeghi, K. Heshmat-Ghahdarijani, M. Talaei, A. Safaei, N. Sarrafzadegan, H. Roohafza, The predictive value of atherogenic index of plasma in the prediction of cardiovascular events; a fifteen-year cohort study, Adv. Med. Sci. 66 (2021) 418–423.
[11] S.H. Kim, Y.K. Cho, Y.J. Kim, C.H. Jung, W.J. Lee, J.Y. Park, et al., Association of the atherogenic index of plasma with cardiovascular risk beyond the traditional risk factors: a nationwide population-based cohort study, Cardiovasc. Diabetol. 21 (2022) 81.
[12] J. Alizargar, C.H. Bai, N.C. Hsieh, S.V. Wu, Use of the triglyceride-glucose index (TyG) in cardiovascular disease patients, Cardiovasc. Diabetol. 19 (2020) 8.
[13] J.R. Ulloque-Badaracco, E.A. Hernandez-Bustamante, E.A. Alarcon-Braga, M. D. Mosquera-Rojas, A. Campos-Aspajo, F.E. Salazar-Valdivia, et al., Atherogenic index of plasma and coronary artery disease: a systematic review, Open Med. (Wars). 17 (2022) 1915–1926.
[14] T. Angkananard, T. Anothaisintawee, M. McEvoy, J. Attia, A. Thakkinstian, Neutrophil lymphocyte ratio and cardiovascular disease risk: a systematic review and Meta-analysis, Biomed. Res. Int. 2018 (2018) 2703518.
[15] P. Salvi, C. Baldi, F. Scalise, A. Grillo, L. Salvi, I. Tan, et al., Comparison between invasive and noninvasive methods to estimate subendocardial oxygen supply and demand imbalance, J. Am. Heart Assoc. 10 (2021), e021207.
[16] K. Tagawa, Y. Choi, S.G. Ra, T. Yoshikawa, H. Kumagai, S. Maeda, Resistance training-induced decrease in central arterial compliance is associated with decreased subendocardial viability ratio in healthy young men. Applied physiology, nutrition, and metabolism =, Physiol. Appl. Nutr. Metab. 43 (2018) 510–516.
[17] A.N. Bennett, D.M. Dyball, C.J. Boos, N.T. Fear, S. Schofield, A.M.J. Bull, et al., Study protocol for a prospective, longitudinal cohort study investigating the medical and psychosocial outcomes of UK combat casualties from the Afghanistan war: the ADVANCE study, BMJ Open 10 (2020), e037850.
[18] A.H. Tzamaloukas, A. Patron, D. Malhotra, Body mass index in amputees, JPEN J. Parenter. Enteral Nutr. 18 (1994) 355–358.
[19] S.Y. Yoong, D. Miles, P.A. McKinney, I.J. Smith, N.J. Spencer, A method of assigning socio-economic status classification to British armed forces personnel, J. R. Army Med. Corps 145 (1999) 140–142.
[20] T. Osler, S.P. Baker, W. Long, A modification of the injury severity score that both improves accuracy and simplifies scoring, J. Trauma 43 (1997) 922–925, discussion 5–6.
[21] G. Pucci, J. Cheriyan, A. Hubsch, S.S. Hickson, P.R. Gajendragadkar, T. Watson, et al., Evaluation of the Vicorder, a novel cuff-based device for the noninvasive estimation of central blood pressure, J. Hypertens. 31 (2013) 77–85.
[22] G.D. Buckberg, D.E. Fixler, J.P. Archie, J.I. Hoffman, Experimental subendocardial ischemia in dogs with normal coronary arteries, Circ. Res. 30 (1972) 67–81.
[23] Y. Shahin, H. Barakat, R. Barnes, I. Chetter, The Vicorder device compared with SphygmoCor in the assessment of carotid-femoral pulse wave velocity in patients with peripheral arterial disease, Hyperten. Re. : Off. J. Japan. Soc. Hypertens. 36 (2013) 208–212.
[24] Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ)-Short and Long Forms, 2005.
[25] C.L. Craig, A.L. Marshall, M. Sjöström, A.E. Bauman, M.L. Booth, B.E. Ainsworth, et al., International physical activity questionnaire: 12-country reliability and validity, Med. Sci. Sports Exerc. 35 (2003) 1381–1395.
[26] D. Tsiachris, C. Tsioufis, D. Syrseloudis, D. Roussos, I. Tatsis, K. Dimitriadis, et al., Subendocardial viability ratio as an index of impaired coronary flow reserve in hypertensives without significant coronary artery stenoses, J. Hum. Hypertens. 26 (2012) 64–70.
[27] J.P. Noon, T.C. Trischuk, S.A. Gaucher, S. Galante, R.L. Scott, The effect of age and gender on arterial stiffness in healthy Caucasian Canadians, J. Clin. Nurs. 17 (2008) 2311–2317.
[28] J. Czernin, P. Müller, S. Chan, R.C. Brunken, G. Porenta, J. Krivokapich, et al., Influence of age and hemodynamics on myocardial blood flow and flow reserve, Circulation. 88 (1993) 62–69.
[29] A. Jekell, M. Kalani, T. Kahan, Skin microvascular reactivity and subendocardial viability ratio in relation to dyslipidemia and signs of insulin resistance in non-diabetic hypertensive patients, Microcirculation. 29 (2022), e12747.
[30] R.J. Doonan, P. Scheffler, A. Yu, G. Egiziano, A. Mutter, S. Bacon, et al., Altered arterial stiffness and subendocardial viability ratio in young healthy light smokers after acute exercise, PLoS One 6 (2011), e26151.
[31] S.A. Nabipoorashrafi, S.A. Seyedi, S. Rabizadeh, M. Ebrahimi, S.A. Ranjbar, S. K. Reyhan, et al., The accuracy of triglyceride-glucose (TyG) index for the screening of metabolic syndrome in adults: a systematic review and meta-analysis, Nutr. Metab. Cardiovasc. Dis. 32 (2022) 2677–2688.
[32] D.H. Son, H.S. Lee, Y.J. Lee, J.H. Lee, J.H. Han, Comparison of triglyceride-glucose index and HOMA-IR for predicting prevalence and incidence of metabolic syndrome, Nutr. Metab. Cardiovasc. Dis. 32 (2022) 596–604.
[33] I.J. Neeland, R. Ross, J.P. Després, Y. Matsuzawa, S. Yamashita, I. Shai, et al., Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement, Lancet Diabetes Endocrinol. 7 (2019) 715–725.
[34] R.N. Bellet, L. Adams, N.R. Morris, The 6-minute walk test in outpatient cardiac rehabilitation: validity, reliability and responsiveness–a systematic review, Physiotherapy. 98 (2012) 277–286.
[35] S.D. Navaneethan, J.P. Kirwan, E.M. Remer, E. Schneider, B. Addeman, S. Arrigain, et al., Adiposity, physical function, and their associations with insulin resistance, inflammation, and Adipokines in CKD, Am. J. Kidney Dis. 77 (2021) 44–55.
[36] J. Tay, A.M. Goss, J.L. Locher, J.D. Ard, B.A. Gower, Physical function and strength in relation to inflammation in older adults with obesity and increased Cardiometabolic risk, J. Nutr. Health Aging 23 (2019) 949–957.
[37] P.H. Lee, D.J. Macfarlane, T.H. Lam, S.M. Stewart, Validity of the international physical activity questionnaire short form (IPAQ-SF): a systematic review, Int. J. Behav. Nutr. Phys. Act. 8 (2011) 115.
[38] H. Sakuta, T. Suzuki, Rank in self-defense forces and risk factors for atherosclerotic disease, Mil. Med. 170 (2005) 820–823.